The integrin I domain: crystals, metals and related artefacts

Robert Liddington* and Laurie Bankston

Address: Department of Biochemistry, University of Leicester, Leicester LE1 7RH, UK.

*Corresponding author.
E-mail: rcl6@le.ac.uk

Structure 15 July 1998, **6**:937–938
http://biomednet.com/elecref/0969212600600937

All protein crystals are artefacts and contain artefacts, in the common and scientific senses of that word. Indeed, the crystal lattice can be thought of as an allosteric effector that locks a protein into one particular conformation, selecting a state that may not predominate in solution. In this way, the lattice has been put to good use in the study of allosteric systems [1].

The integrins are a family of heterodimeric plasma membrane proteins that bind the extracellular matrix or counter receptors on other cells. The adhesiveness of their extracellular domains is allosterically controlled by binding events in their cytoplasmic domains that trigger conformational changes across the plasma membrane. In early 1995, we published the first crystal structure of an integrin domain, the so-called A or I domain from CD11b [2]. The domain contained a Mg^{2+} ion coordinated by three loops on its upper surface. A glutamate sidechain from a second I domain in the crystal completed the octahedral coordination sphere around the metal ion. Given the Mg^{2+} dependence of ligand binding by I domains and the existence of critical glutamates and aspartates in the binding motifs of most integrin ligands (such as intercellular adhesion molecule [ICAM]), we suggested that the crystal contact might be a mimic of an authentic integrin–ligand complex. We therefore called it the metal ion dependent adhesion site or MIDAS motif. Since that time, no crystal structure of an I-domain–ligand complex has been published, but mutagenesis work has confirmed our suggestion that the upper surface of the domain including the MIDAS motif and surrounding area is important in ligand binding [3].

Later in 1995, we published a second crystal form of the CD11b I domain, this time grown under similar conditions but at 4°C rather than at room temperature and in the presence of Mn^{2+} rather than Mg^{2+} [4]. This new crystal form did not include a crystal contact resembling a ligand mimetic, and other details of the metal coordination had changed. In particular, a threonine residue, that is critical for metal-dependent ligand binding in all integrin I domains [5], no longer bound the metal directly, although an aspartic acid residue from another loop became directly bound. These subtle changes in metal stereochemistry were linked to a large downward shift of the C-terminal helix by ~10 Å, which altered the shape of the ligand-binding surface and exposed two buried phenylalanine sidechains to solvent. We pointed out that these structural changes were intriguingly similar to those seen in the G proteins in which, on exchanging GDP for GTP, a threonine sidechain gains a direct bond to Mg^{2+}, leading to the exposure of a hydrophobic effector-binding motif [6].

We suggested, by analogy with the G proteins, that the Mg^{2+} crystal form might represent the high affinity, ligand-bound ('active') form of the I domain and the Mn^{2+} crystal the low affinity ('inactive') form. This posed a problem for some of the integrin cognoscenti, as Mn^{2+} is well known as an activator of integrins *in vitro*. As noted at the time, however, the choice of metal ion could not be the sole determinant of protein conformation, as we had grown crystals of another I domain, from CD11a, in the presence of both Mg^{2+} and Mn^{2+} and obtained an identical protein conformation in both cases, similar to the Mn^{2+}-bound CD11b I domain (later confirmed by Qu and Leahy [7]). Rather, we argued that it was the presence of the ligand mimetic that was the decisive factor in driving the conformation into the active state, a state that may have been favoured by the higher temperature and higher cation concentration employed in the case of Mg^{2+}. One possibility is that the less stable, active, conformer is present in solution a smaller percentage of the time at the lower temperature and is thus more difficult to capture in the lattice. Our attempts to grow the Mn^{2+} form of CD11b under conditions similar to the Mg^{2+} form were frustrated by the instability of aqueous Mn^{2+} at room temperature and alkaline pH. To date, the structures of three different I domains grown under six different conditions of cation have been published [2,4,7–9]. It turns out that our original CD11b Mg^{2+}-bound I domain is the odd-man-out; all the other crystal structures have a consistent conformation equivalent to our inactive conformer, and none of them contain a ligand mimetic.

In this issue of *Structure*, Baldwin and colleagues describe a new crystal form of the CD11b I domain. First, they grew crystals in the absence of metal, and found that the protein conformation was very similar to our Mn^{2+}-bound (inactive) structure, as previously observed for metal-free CD11a I domain [7]. They next soaked metal-free crystals in various cation solutions, and found that Mn^{2+}, Mg^{2+} and Cd^{2+}, but not Ca^{2+}, bound to the MIDAS motif. Metal binding induced no significant changes in the protein, and they suggest that this argues against our model of activation. In fact, their data do not contradict our model. As we

argued that the presence of the ligand mimetic was the deciding factor in determining conformation, in the absence of a ligand mimetic we would not expect to see any changes in structure on binding metal, and the crystal lattice would prevent any structural rearrangements that could create a ligand mimetic. Thus, the lack of structural changes under these conditions tells us nothing about the *possibility* of structural changes, and neither supports nor refutes our model; only an authentic I-domain–ligand complex crystal structure can do that. Baldwin *et al.* also appear to challenge our suggestions for the role of Ca^{2+}, and we would like to clear up some confusion here. That Ca^{2+} does not *support* I-domain–ligand binding is well established, and is clearly a consequence of poor binding of Ca^{2+} to the I domain [10]. In our 1995 paper, we suggest why this is so: 'Mg^{2+} and Mn^{2+} can be stably coordinated by uncharged serine and threonine residues (owing to their smaller size), but Ca^{2+} cannot be thus ligated'. In fact, strong Ca^{2+} binding requires at least two ligands carrying formal negative charges. As a separate point, we suggested how Ca^{2+} might *inhibit* ligand binding: although a weak binder, Ca^{2+} would bind better to the inactive conformer, owing to the availability of an aspartic acid for direct coordination. Thus, very high concentrations of Ca^{2+} could in principle inhibit Mg^{2+}-dependent ligand binding by binding preferentially to the inactive conformer and stabilizing it.

In summary, our model, in which tertiary 'shape-shifting' in the I domain creates a ligand-binding surface, remains both unproven and unrefuted. It is still possible that activation of the I domain entails no more than a simple 'unmasking' of a pre-existing ligand-binding surface via quaternary changes in the integrin. Baldwin *et al.* propose a third possibility, that activation is a simple consequence of Mg^{2+} binding. For this to provide a mechanism of regulation *in vivo* it requires that extracellular magnesium concentrations be regulated, but there is no evidence for this in plasma, where the leukocyte integrins operate, and where the Mg^{2+} concentration is sufficient to ensure full occupancy of the I domain at all times.

As a footnote, Baldwin *et al.* suggest that our active conformation is 'likely to be a construct artefact'. It is not, at least not in the sense intended. The protein was identical in the two crystal forms, beginning at residue Gly127 and finishing at residue Ala318, with six residues from the expression vector attached to the C terminus. As crystals grown in the presence of Mn^{2+} showed ordered structure as far as Ala318, the Mg^{2+}-bound form cannot, as suggested by Baldwin *et al.*, be truncated at Lys315.

References

1. Perutz, M.F. (1989). Mechanisms of cooperativity and allosteric regulation in proteins. *Q. Rev. Biophys.* **22**, 139-226.
2. Lee, J.-O., Rieu, P., Arnaout, M.A. & Liddington, R.C. (1995). Crystal structure of the A-domain from the α subunit of integrin CR3 (CD11b/CD18). *Cell* **80**, 631-635.
3. Huang, C. & Springer, T.A. (1995). A binding interface on the I domain of lymphocyte function associated antigen-1 (LFA-1) required for specific interaction with intercellular adhesion molecule 1 (ICAM-1). *J. Biol. Chem.* **270**, 19008-19016.
4. Lee, J.-O., Bankston, L.A., Arnaout, M.A. & Liddington, R.C. (1995). Two conformations of the integrin A-domain (I-domain): a pathway for activation? *Structure* **3**, 1333-1340.
5. Kamata, T., Wright, R. & Takada, Y. (1995). Critical threonine and aspartate residues within the I-domains of β2 integrins for interactions with intercellular adhesion molecule 1 (ICAM-1) and C3bi. *J. Biol. Chem.* **270**, 12531-125355.
6. Pai, E.F., Krengel, U., Petsko, G.A., Goody, R.S., Kabsch, W. & Wittinghofer, A. (1990). Refined crystal structure of the triphosphate conformation of H-ras p21 at 1.35 Å resolution: implications for the mechanism of GTP hydrolysis. *EMBO J.* **9**, 2351-2359.
7. Qu, A. & Leahy, D.J. (1996). The role of the divalent cation in the structure of the I domain from the CD11a/CD18 integrin. *Structure* **4**, 931-942.
8. Qu, A. & Leahy, D.J. (1995). Crystal structure of the I-domain from the CD11a/CD18 (LFA-1, aLb2) integrin. *Proc. Natl Acad. Sci. USA* **92**, 10277-10281.
9. Emsley, J., King, S., Bergelson, J. & Liddington, R. (1997). Crystal structure of the I domain from integrin α2β1, *J. Biol. Chem.* **272**, 28512-28517.
10. Michishita, M., Videm, V. & Arnaout, M.A. (1993). A novel divalent cation-binding site in the A domain of the β2 integrin CR3 (CD11b/CD18) is essential for ligand binding. *Cell* **72**, 857-867.